

IN THE MATTER OF European
Patent 0181150 in the name
of Chiron Corporation AND
oppositions thereto under
Article 100 EPC.

Affidavit of John Alexander Thomas Young

I, John Alexander Thomas Young, of 388 Lexington Street, Newton, MA 02166 do hereby solemnly and sincerely state as follows:

1. I hold the position of Assistant Professor, Dept. of Microbiology and Molecular Genetics, Harvard Medical School. My current research interest is to understand the mechanism of retroviral entry into cells. In addition to my research responsibilities, I teach in the microbiology graduate student course and I am co-director of the Virology 200 course at Harvard Medical School.
2. My curriculum vitae is attached hereto as Exhibit JATY-1.
3. I have been provided with and have considered the following documents:
 - i) Declaration of Timothy French
 - ii) 867501 US patent application 867501 (31st October 1984)
 - iii) European Patent 0181 150 B1
4. I have been asked to comment upon the criticisms made of document ii) by Dr French in his declaration, to provide my understanding of the level of cross-reactivity between HTLV-I and -II with HIV-1 and to define my understanding of the term "vaccine".

SV40 Expression Systems

5. Before October 1984, the SV40 genome was already cloned and was well characterized. Important elements of the viral genome required for early and late mRNA synthesis and for initiating DNA replication were identified. This information was readily available to those skilled in the art.
 - a) The entire DNA sequence of the SV40 genome was already known (Flers, Nature 273, 113; Reddy, Science 200, 494).
 - b) The origin of DNA replication was localized to a region of 85 nucleotides within the SV40 genome (Subramanian Nuc. Acids. Res. 5, 3635-3642).
 - c) The SV40 early promoter region was defined and important regulatory elements within this promoter were identified (e.g. Benoist, Nature 290, 304; Everett, Nuc. Acids Res. 11, 2447; Fromm, J. Mol. and App. Genet. 1, 457; Dynan, Cell 35, 79)
 - d) Plasmid vectors containing SV40 transcriptional control elements for expression of heterologous genes in eukaryotic cells were described (e.g. Mulligan, Science 209, 1422; Mulligan, Mol. Cell. Biol. 1, 449; Subramani Mol. Cell. Biol. 1, 854). A prominent review article published in 1981

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described the features of these SV40-based expression vectors (Elder, Ann Rev. Genet. 15, 295).

6. With the detailed knowledge of the important elements of the SV40 genome, and with the availability of cloned SV40 DNA elements in a number of labs, it would have been a trivial exercise for those skilled in the art to isolate DNA fragments containing the SV40 origin of replication and the early promoter region for the construction of plasmid expression vectors.

SacI Site in Plasmid pSV-7c/7D

7. Dr. French points out that the application does not provide an explanation for a SacI site within plasmid pSV-7e. On page 28, line 8, the application states that the pSV-7e plasmid was derived from plasmid pSV-7c (which does not contain the SacI restriction enzyme site). However, inspection of Fig. 5 reveals that the pSV-7c/7D plasmid (derived from pSV-7c) contains a SacI restriction enzyme site located upstream of the Env open reading frame (Fig. 4). Obviously there was a typographical error in the application, and plasmid pSV-7c/7D was used to construct plasmid pSV-7e.

Linker Details

8. Dr. French states that, by following the teachings of the application, one skilled in the art would have attempted to ligate a blunt-ended double-stranded oligonucleotide (page 27, line 22) to StuI/XbaI-digested pSV-7b. Dr. French further states that one skilled in the art would interpret the labels 5' and 3' (page 27, line 22) to synthesize a double-stranded oligonucleotide lacking the restriction enzyme sites described on page 27, line 22 and in Fig. 5.
9. I find it inconceivable that one skilled in the art would interpret the teachings of the application in the manner suggested by Dr. French. Specifically:
 - a) an individual skilled in the art would be able to determine that the restriction enzyme sites (page 27, line 22 and Fig. 5) were important for subsequent cloning steps.
 - b) an individual skilled in the art would recognize the BglII site (AGATCT), the EcoRI site (GAATTC), the SmaI site (CCCGGG), and the KpnI site (GGTACC) contained within the double stranded oligonucleotide, and would recognize the site for XbaI (TCTAGA).
 - c) one skilled in the art would know that the double-stranded oligonucleotide (page 27, line 22) contains an incomplete XbaI site: the sequence at the right end of the bottom DNA strand (GATC) corresponds to the overhang that remains following restriction enzyme cleavage of DNA by XbaI.

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Based on a) to c) above, one skilled in the art would correctly conclude that the 5' and 3' labels on the upper strand of this oligonucleotide were inverted in the application and would construct the following double-stranded oligonucleotide:

5'- AGATCTCGAATTCCCCGGGGGTACCT - 3'
TCTAGAGCTTAAGGGGCCCCCATGGAGATC

This reagent would be expected to ligate to a plasmid vector cut with *Stu*I (blunt-end) and *Xba*I (sticky-end) and as a consequence would introduce the desired restriction enzyme sites into the recombinant plasmid.

Immunofluorescence Experiments

10. The application describes immunofluorescence experiments performed with COS-7 cells transiently-transfected with plasmid pSV-7c/7D encoding ARV-2 Env (page 25 line 19) and with a plasmid encoding ARV-2 Gag (page 27, line 32). These cells were incubated with AIDS patient sera and with control normal human sera and with fluoresceinated goat anti-human sera (page 25, lines 29 to 32). Approximately 5% of the cells transfected with pSV-7c/7D showed bright immunofluorescence with AIDS patient sera but not with control human sera (page 26). Similarly, approximately 5% of the cells transfected with the ARV-2 Gag expression plasmid exhibited bright immunofluorescence when incubated with the AIDS sera (page 28). Cells transfected with a control plasmid did not show immunofluorescence with any of the sera (pages 26 and 28). The numbers of immunofluorescent cells (5%) described for both of these cell populations closely approximates the number of cells transfected by this procedure (i.e. 6%; Parker J. Virol. 31, 360).
11. These are precisely the results expected if the plasmids encoding either ARV-2 Gag or Env generated, in transfected cells, proteins that are specifically reactive with AIDS sera.

HTLV-I and II

12. Experiments performed by the Gallo and Essex groups led to the wrong conclusion that HIV-1 was significantly similar to the human T cell leukemia viruses, HTLV-I and HTLV-II. The bases for this claim were that these viruses apparently shared some features including significant antigenic cross-reactivity, significant genome cross-hybridization and organization, and a similar morphological appearance (e.g. Arya Science 225, 927; Schupbach, Science 224, 503; Gallo Science 224, 500; Hahn, Nature 312, 166; Essex, Science 220, 859; Essex, Science 221, 1061). Other groups later found no such similarity between HIV-1 and the two types of HTLV (e.g. Barre-Sinoussi, Science 220, 868; Sanchez-Pescador, Science 227, 484; Alizon, Nature 312, 757).
13. HIV-1 is no more closely related to HTLV-I and HTLV-II than it is to other retroviruses that infect chickens or mice

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(Sanchez-Pescador, Science 227, 484). Additional experiments by others, and by the Gallo group confirmed that HIV-1, a lentivirus, is much more closely related to a sheep lentivirus (visna virus), than it is to either HTLV-I and HTLV-II (e.g. Gonda, Science 227, 173).

Vaccine - Definition

14. My understanding of the term "vaccine" is a viral preparation or viral product that when introduced into an individual generates humoral and/or cellular immunity and confers protection against virus-induced disease.

Further affiant sayeth not.

Name: John Young
Signature: *John Young*....
Date: 10/29/96

Sworn before me this day the 25th day of October 1996

Name: *Pauline H. Bergman*
Signature: *Pauline H. Bergman* (Notary Public)
Date: October 27, 1996

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